A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP3759.

Long-Term PM_{10} Exposure and Cause-Specific Mortality in the Latium Region (Italy): A Difference-in-Differences Approach

Matteo Renzi,[1] Francesco Forastiere,[2,3] Joel Schwartz,[4] Marina Davoli,[1] Paola Michelozzi,[1] and Massimo Stafoggia[1,5]

[1]Department of Epidemiology, Lazio Region Health Service/ASL Roma 1, Rome, Italy
[2]Institute of Biomedicine and Molecular Immunology (IBIM), National Research Council, Palermo, Italy
[3]Environmental Research Group, King's College, London, UK
[4]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[5]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Background: The link between particulate matter (PM) exposure and adverse health outcomes has been widely evaluated using large cohort studies. However, the possibility of residual confounding and lack of information about the health effects of PM in rural and suburban areas are unsolved issues.

Objective: Our aim was to estimate the effect of annual PM $\leq 10\,\mu m$ (PM_{10}) exposure on cause-specific mortality in the Latium region (central Italy, of which Rome is the main city) during 2006–2012 using a difference-in-differences approach.

Methods: We estimated daily PM_{10} concentrations for each $1\,km^2$ of the region from 2006 to 2012 by use of satellite data, land-use predictors, and meteorological parameters. For each of the 378 regional municipalities and each year, we averaged daily PM_{10} values to obtain annual mean PM_{10} exposures. We applied a variant of the difference-in-differences approach to estimate the association between PM_{10} and cause-specific mortality by focusing on within-municipality fluctuations of mortality rates and annual PM exposures around municipality means, therefore controlling by design for confounding from all spatial and temporal potential confounders. Analyses were also stratified by population size of the municipalities to obtain effect estimates in rural and suburban areas of the region.

Results: In the period 2006–2012, we observed deaths due to three causes: 347,699 nonaccidental; 92,787 cardiovascular; and 16,509 respiratory causes. The annual average (standard deviation, SD) PM_{10} concentration was $21.9\,(\pm 4.9)\,\mu g/m^3$ in Latium. For each $1\text{-}\mu g/m^3$ increase in annual PM_{10} we estimated increases of 0.8% (95% confidence intervals (CIs): 0.2%, 1.3%), 0.9% (0.0%, 1.8%), and 1.4% (−0.4%, 3.3%) in nonaccidental, cardiovascular, and respiratory mortality, respectively. Similar results were found when we excluded the metropolitan area of Rome from the analysis. Higher effects were estimated in the smaller municipalities, e.g., those with population <5,000 inhabitants.

Conclusion: Our study suggests a significant association of annual PM_{10} exposure with nonaccidental and cardiorespiratory mortality in the Latium region, even outside Rome and in suburban and rural areas. https://doi.org/10.1289/EHP3759

Introduction

The association between particulate matter (PM) with aerodynamic diameter $\leq 10\,\mu m$ (PM_{10} exposure and human health has been widely investigated. The *Lancet* Commission on Pollution and Health estimated that ambient PM causes 4.2 million of deaths each year worldwide (Landrigan et al. 2017) on the basis of data produced for the Global Burden of Disease study (Cohen et al. 2017).

An extensive body of epidemiological literature showed adverse effects of long-term exposure to PM_{10} on mortality and morbidity (e.g., Beelen et al. 2014a; Cesaroni et al. 2014; Di et al. 2017; Hoek et al. 2013) in population-based cohort studies. These studies are characterized by recruitment and follow-up of subjects until the occurrence of the study outcome or censoring. Exposure to air pollutants is usually estimated for each subject at the residential address, either at baseline or as a time-varying annual exposure, and the association with the study outcome is investigated by contrasting individual exposures over space (or space and year), while controlling for potential individual-level or area-level risk factors. One of the major criticisms usually directed to cohort studies is the possibility of residual confounding due to unmeasured or mismeasured confounders (Moolgavkar et al. 2017). Moreover, some other issues are related to standard observational studies. Cohorts are population samples that are not always representative of the whole population. For example, both the American Cancer Society (ACS) cohort and the Nurses' Health Study (NHS) cohort examined populations with considerably higher levels of education than average (Pope et al. 1995; Puett et al. 2009) or considered only city dwellers (Lepeule et al. 2012) (Jerrett et al. 2013; Krewski 2009).

The need to develop studies that are able to account for measured or unmeasured confounders in the design phase, rather than in the analysis phase, has become increasingly apparent in the last few years. Estimation of a causal effect would require, in principle, the definition of a counterfactual framework, where the study outcomes under alternative exposure scenarios are compared (Hernán and Robins 2019). However, in observational studies, only one exposure distribution is measured (or estimated) for a population for a given time, and causal inference methods try to find the best surrogates for alternative (unobserved) exposure distributions (Rubin 1991). The application of propensity score models suffers from the same concerns about unmeasured confounders (Stürmer et al. 2005), suggesting the importance of alternative study designs that address omitted confounders. The difference-in-differences approach could be a suitable methods to control confounding by design (Card and Krueger 1994). A study conducted in the United States assessed the long-term effects of fine particulate matter with aerodynamic diameter $\leq 2.5 \leq m$ ($PM_{2.5}$) on mortality in New Jersey (Wang et al. 2016) using a modified version of the difference-in-differences approach by comparing annual changes in mortality rates around census tract–specific means with concurrent annual changes in average exposures within the same census tract. A similar approach was used by Kioumourtzoglou et al. in 207 cities of the northern United States (Kioumourtzoglou et al. 2016).

Address correspondence to Matteo Renzi, Via Cristoforo Colombo 112, 00147, Rome, Italy. Telephone: +39 0699722185. Email: m.renzi@deplazio.it

Supplemental Material is available online (https://doi.org/10.1289/EHP3759).

The authors declare they have no actual or potential competing financial interests.

Received 12 April 2018; Revised 24 April 2019; Accepted 13 May 2019; Published 5 June 2019.

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehponline@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Another limitation common to most studies investigating air pollution health effects is their focus on urban areas, as the availability of environmental data has been usually confined to the main cities. However, rural and suburban areas represent important settings where large portions of the population live. These areas, despite their lower air pollution concentrations, are influenced by different emission sources (Viana et al. 2008), such as biomass combustion, agricultural emissions, and dust from natural sources (Chen et al. 2017; Pey et al. 2013). Established evidence now shows that low concentrations of PM are related to adverse health outcomes (Makar et al. 2017), that fine and coarse particles (PM between 2.5 and 10 μm) display comparable short-term health effects (Zanobetti and Schwartz 2009), and that PM from emission sources other than vehicular traffic (such as desert dust advections and biomass burning) are also related to increased mortality and morbidity (Kim et al. 2013; Stafoggia et al. 2015). In the last few years, data from satellite observations have been made available providing relevant information to supplement ground-level measurements (Kloog et al. 2011, 2014). These data have been used recently in Italy to estimate daily PM_{10} concentration at 1-km^2 spatial resolution for the period 2006–2012 (Stafoggia et al. 2017).

The objective of this study was to assess the association between long-term exposure to PM_{10} and cause-specific mortality (nonaccidental, cardiovascular, and respiratory) in the Latium region (central Italy), in the 2006–2012 period by replicating the difference-in-differences approach developed by Wang et al. (Wang et al. 2016). In addition, we aimed to evaluate differential effects of PM on cause-specific mortality in urban, suburban, and rural areas of the region.

Methods

Study Area

The Latium region is in central Italy; it is 17,242 km^2 wide, is divided into 378 municipalities, and had a total population of 5,304,778 inhabitants in 2006 (Figure 1). Rome is the major conurbation of the region, with 2,547,677 residents and an area of 1,287 km^2. For the purposes of this study, Rome was further divided into 155 urban zones (Figure 2). For each municipality of Latium and the urban zone of Rome (a total of 532 units of observations), we collected annual population data from the National Institute of Statistics (ISTAT) (www.demo.istat.it), which we used as denominators in the statistical analyses described below.

Health Data

We collected information on mortality data from the Regional Register of Causes of Deaths. For each municipality of the Latium region and each urban zone of Rome, we calculated annual counts of deaths (age 35 + years) from nonaccidental (WHO 1978) (*International Classification of Diseases Ninth*

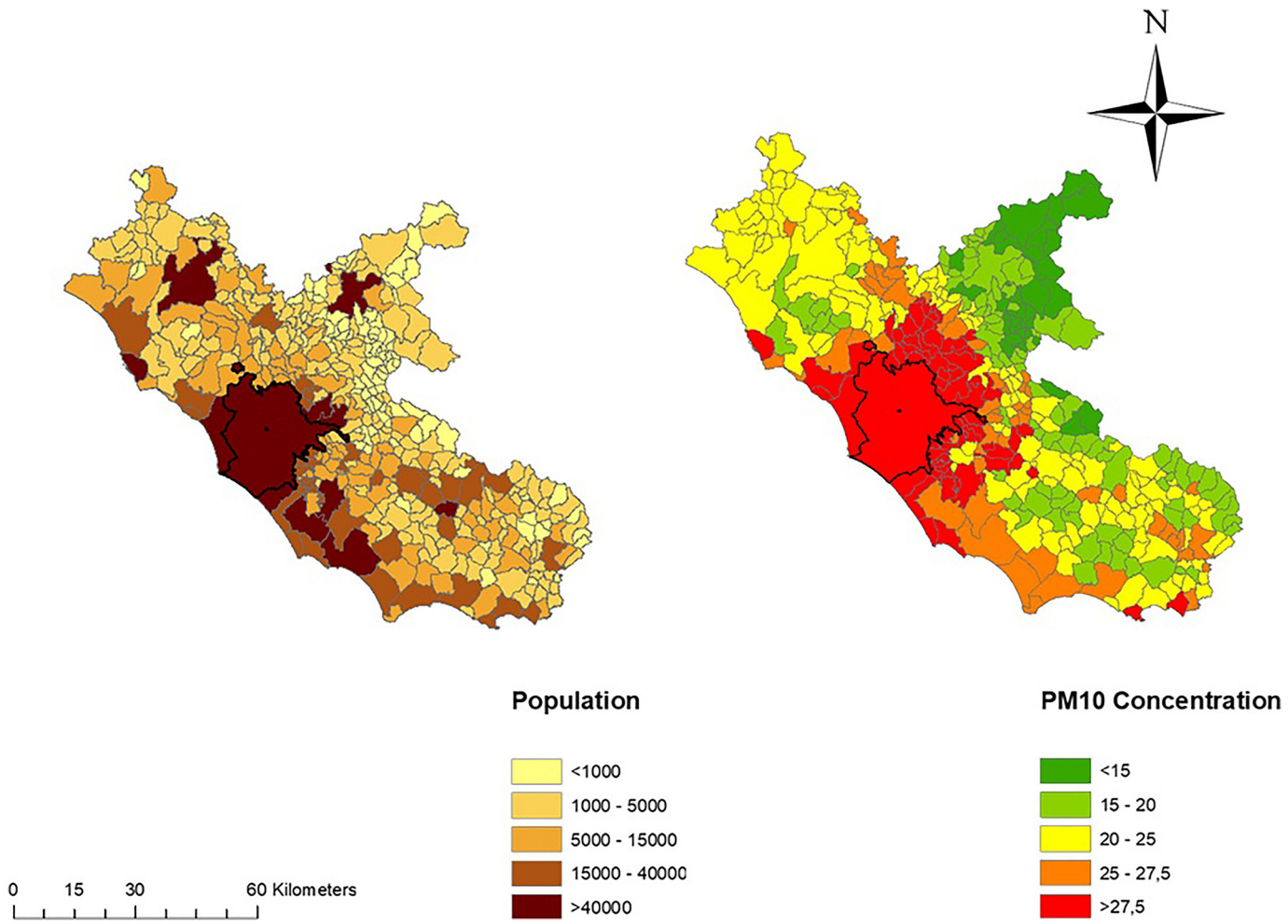

Figure 1. Population size and PM_{10} concentration in 378 municipalities of the Latium Region during the study period. The population size is reported for the year 2006, and the PM_{10} concentration is the average in the whole period.

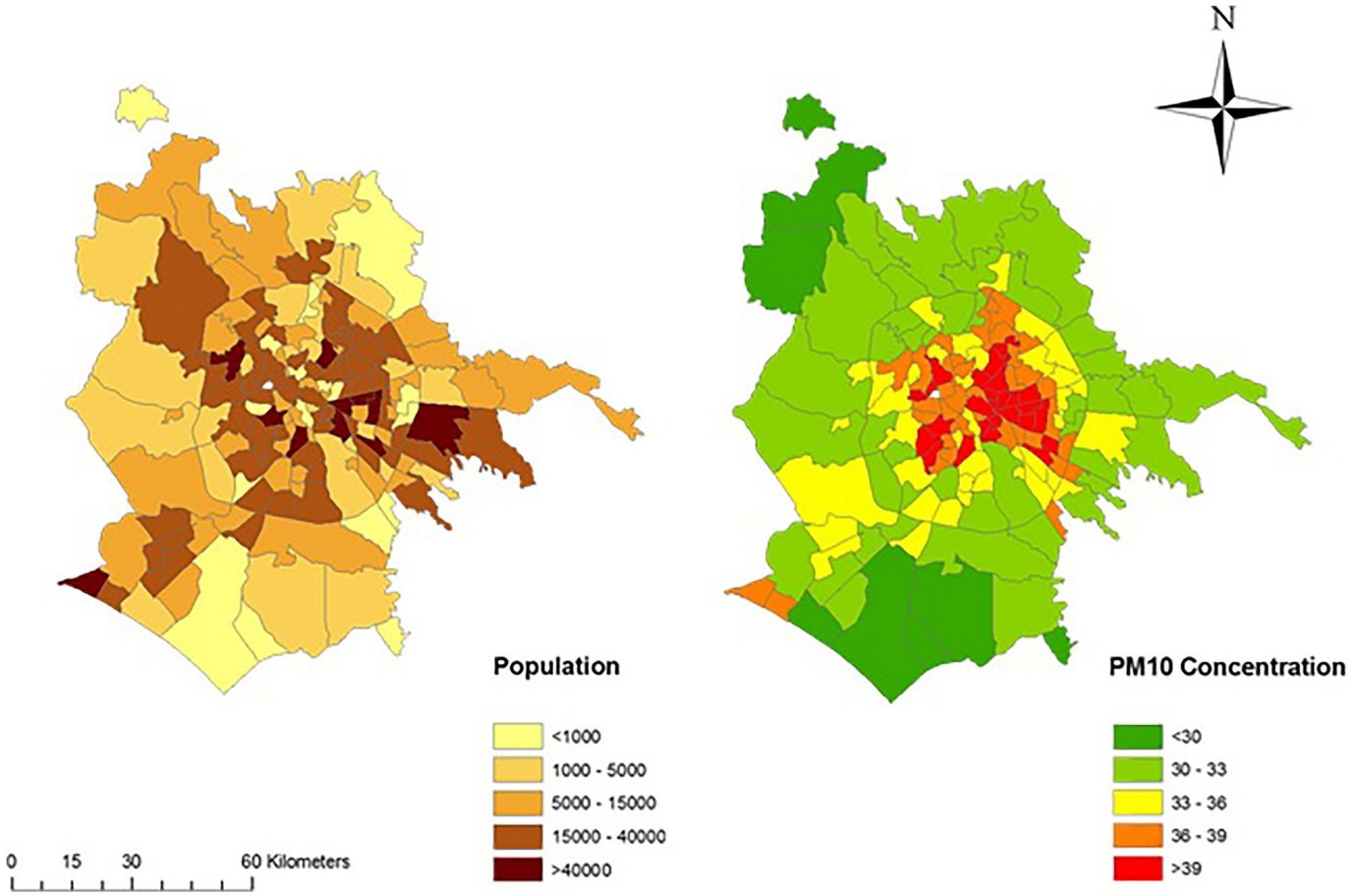

Figure 2. Population size and PM_{10} concentration in 155 urban zones of Rome during the study period. The population size is reported for the year 2006, and the PM_{10} concentration is the average in the whole period.

Revision (ICD-9; all codes: 0–799), cardiovascular (CVD; codes 390–459), and respiratory causes (Resp; codes 460–519) during the study period (2006–2012).

Exposure Assessment

Daily PM_{10} concentrations were estimated for each municipality and urban zone and each year on the basis of a spatiotemporal model developed for the entire nation of Italy (Stafoggia et al. 2017). Briefly, we collected multiple sources of data on spatial and spatiotemporal parameters, such as land use and meteorological for each day in 2006–2012 and each square kilometer of Italy, and developed a three-stage model to predict daily PM_{10} values for each 1×1-km grid cell and day by calibrating the satellite aerosol optical depth, land use, and meteorological terms to PM_{10} monitors. We then computed annual PM_{10} for each spatial unit of interest (municipality for the Latium region, urban zone for Rome) by averaging the predicted daily PM_{10} estimates over all grid cells intersecting the spatial units and weighting for the area of intersection (Figure S1 shows the grid cells and the units of the study).

We obtained daily estimates of air temperature by the ERA-Interim reanalysis project (Dee et al. 2011), released by the European Centre for Medium-Range Weather Forecasts (ECMWF). We collected data about air temperature estimated at a 2-meter height at 0000 hours (12:00 P.M.) and 12000 hours (12:00 A.M.) with spatial resolution of $0.125° \times 0.125°$ (approximately 10×10 km). We calculated a daily mean value of temperature by averaging the two daily retrievals. For the purposes of the study, we considered mean summer temperature (from June to August), mean winter temperature (from December to February), and their standard deviations (SDs). For each spatial unit, mean (and SDs) temperatures were computed by averaging the daily values over all grid cells within the spatial units.

Statistical Analysis

We applied a variant of the difference-in-differences approach to assess the relationship between annual PM_{10} and cause-specific mortality.

The essence of the design is that differences in concentrations across time in a given location (municipality or zone) are related to differences in rates of death in the same municipality or zone so that the role of temporally stable individual and behavioral factors (including unmeasured ones) are canceled out because the comparisons are occurring within the same populations (Card and Krueger 1994). Temporally variable confounders are controlled by contrasting this relation of difference across municipalities or zones with the relation of differences in time in other municipalities (with different exposure differences), thereby controlling for time trends in confounders that are similar across the study region. The standard version of the difference-in-differences approach was based on the comparison between two different areas in two different years (Card and Krueger 1994). Recently, Wang et al. (2016) developed a variant of that study design. The method has been generalized to the case of multiple spatial units and time periods (Wang et al. 2016), by introducing in the statistical model indicator variables for each spatial unit and each time period to remove confounding by all known and unknown factors varying across areas (but

considered fixed in time) and over years (but homogenous across space), respectively. Potential confounders, thus, are those factors that: *a*) display different time variations across the study areas, and *b*) are correlated with the exposure (annual fluctuations of PM_{10} around the spatial unit average in this study). In the main analysis, we assumed that the only variable with such characteristics might be air temperature, as it seems implausible that, for example, variations from year to year in smoking rates in a municipality or zone around the overall mean for that area and the annual fluctuations in Latium as a whole are correlated with variations from year to year in particle concentrations in that area, around its area specific mean and the overall year-to-year difference in Latium. Therefore, we fit the following model:

$$\ln[E(Y_{s,t})] = \beta_0 + \beta_1 I_s + \beta_2 I_t + \beta_3 Temp_{sum} + \beta_4 Temp_{win} +$$

$$\beta_5 SD(Temp_{sum}) + \beta_6 SD(Temp_{win}) + \beta_7 PM_{10\,s,t} + \ln(P_{s,t})$$

where:

- $Y_{s,t}$ represents the number of deaths in spatial-unit s (378 municipalities of Latium and 155 urban zones of Rome), year t (7-y period, 2006–2012);
- $PM_{10\,s,t}$ is the annual mean concentration of PM_{10} in unit s, time t;
- $P_{s,t}$ is an offset term which represents person-years at risk in unit s, time t;
- I_s is a dummy variable for each spatial unit s;
- I_T is a dummy variable for each year.

Furthermore:

- β_0 is the intercept term;
- β_1, β_2 are regression coefficients adjusting for confounding induced by factors varying across spatial units (and considered fixed in time) (β_1), and over time (homogeneously across the study area) (β_2);
- β_3, β_4, β_5, and β_6 are the regression coefficients for the effects of mean summer and winter temperatures and their standard deviations, respectively; and
- β_7 represents the effect of PM_{10}.

We used the conditional Poisson regression models to analyze the association above described, using the "gnm" package (Turner and Firth 2015). Briefly, we took advantage of conditional Poisson models parameters conditioning on spatial units and "eliminating" (from "eliminate" option of gnm models) the estimates of the variables that did not contribute to the likelihood. Moreover, conditional Poisson models were perfectly comparable with unconditional ones to account for overdispersion and autocorrelation of time-series data (Armstrong et al. 2014).

Because the second objective of our study was disentangling PM effects across different spatial units, and particularly outside of large urban zones cores, we analyzed the whole Latium region and Rome in different ways. First, we analyzed all municipalities of Latium including and excluding Rome as a single unit; then, we focused only on Rome by considering its 155 urban zones; third, we classified municipalities according to their population [1,000–5,000 (194 municipalities); 5,000–14,000 (123); 14,000–40,000 (108); >40,000 inhabitants (31)], and provided PM effects for each group of municipalities. The choice of the cutoff points was made considering the distribution of city-specific populations in the whole Latium region. We decided to apply cutoff points to have a homogeneous representation of the municipalities of Latium in each class.

As sensitivity analysis, we tested the effect of the same year and lagged exposure (lag 0–1) averaging the concentrations in the current and 1 y before. We modeled the summer and winter temperatures with natural splines with 3 and 2 degrees of freedom, respectively, to check the nonlinearity of the association between temperatures and mortality (data not shown).

Finally, we provided alternative approaches for confounding adjustment. Specifically, we assumed that variables might exist that displayed different linear time trends across areas, possibly co-varying with air pollution levels. To adjust for them, we applied mixed models by adding a random intercept for municipality and a random slope by year. Similarly, we ran a fixed effects model where we inserted an interaction term between year (as linear term) and municipality, aimed at capturing residual confounding from other spatiotemporal covariates not accounted for in the main approach.

All results are expressed as percent increases of risk (IR%), and relative 95% confidence intervals (95% CI) per 1-$\mu g/m^3$ increase in annual PM_{10}. All statistical analyses were conducted using R software (version 3.1.2; R Development Core Team), specifically the package "gnm" (Turner and Firth 2015) for the

Table 1. Environmental and mortality data in the Latium region (378 municipalities) and in the urban zones of Rome (155 units) over the period 2006–2012.

				Percentiles				
Latium region	Mean	SD	Min	25th	50th	75th	Max	IQR*
Cause-specific mortality								
Nonaccidental	131.41	1317.54	0	5	15	38	26,987	33
Cardiovascular	35.07	349.64	0	1	5	12	7,032	11
Respiratory	6.24	64.84	0	0	0	2	1,354	2
Environmental data								
PM_{10} ($\mu g/m^3$)	21.91	4.87	9.11	18.62	22.25	25.50	33.81	6.88
Mean winter Temperature (°C)	11.91	3.25	5.43	8.63	13.69	14.73	17.19	6.11
Mean Summer Temperature (°C)	17.81	3.67	12.10	14.62	15.91	21.59	25.20	6.97
Standard deviation of winter T (°C)	2.73	0.72	1.07	2.12	2.79	3.24	4.25	1.12
Standard deviation of summer T (°C)	3.86	1.12	1.02	2.83	4.33	4.82	5.51	1.99
Urban zones of Rome								
Cause-specific mortality								
Nonaccidental	134.75	132.67	0	27	97	194	650	167
Cardiovascular	53.79	54.19	0	11	39	77	264	66
Respiratory	8.61	9.09	0	2	6	13	49	4
Environmental data								
PM_{10} ($\mu g/m^3$)	31.66	3.81	22.84	28.67	31.20	34.44	43.09	5.77
Mean winter Temperature (°C)	12.01	2.59	6.99	9.93	13.13	14.30	15.92	4.37
Mean Summer Temperature (°C)	19.10	3.78	13.83	15.76	16.93	23.53	24.87	7.76
Standard deviation of winter T (°C)	0.63	0.45	0	0.09	0.65	1.01	1.73	0.92
Standard deviation of summer T (°C)	0.88	0.40	0	0.58	0.89	1.24	1.82	0.66

*Interquartile range = 75th–25th percentiles.

main analysis and "lme4" (Bates et al. 2015) for the mixed models.

Results

In the period 2006–2012, we observed deaths due to three causes: 347,699 nonaccidental; 92,787 cardiovascular; and 16,509 respiratory (35 + years) in the entire Latium region. Corresponding figures for Rome were 178,557; 47,437; and 8,772 deaths from those three causes. Table 1 shows the distribution of deaths, PM_{10} and temperature variables in the whole period both in the municipalities of Latium and in the urban zones of Rome. The average PM_{10} concentration during the study period across all the study units was $21.9 \pm 4.9\,\mu g/m^3$ in Latium and $31.7 \pm 3.8\,\mu g/m^3$ in Rome, with interquartile ranges (IQRs) of 6.9 and $5.8\,\mu g/m^3$ respectively. The spatial distribution of population data (year 2006) and PM_{10} concentrations (mean of 2006–2012) are reported in Figure 1 (for Latium) and Figure 2 (for Rome). Population size was represented as total number of residents in the 2006 because no significant changes occurred during the subsequent six years.

Table 2 describes the fluctuations around the area-level mean for PM_{10} annual concentrations and cause-specific mortality rates in Latium (378 municipalities) and Rome (155 urban zones) over the period 2006–2012. Figure 3 describes the SD of the annual PM_{10} concentrations over the study period across the units in Latium.

The results of the main analysis are reported in Table 3. We found positive associations between long-term exposure to PM_{10} and cause-specific mortality in the whole Latium region, with IR% (95% CI) of 0.75% (0.17, 1.34), 0.93% (0.03, 1.83), and 1.42% (−0.38, 3.25) per 1-$\mu g/m^3$ increase in PM_{10}, for nonaccidental, cardiovascular, and respiratory causes, respectively. In Rome, we found a significant PM_{10} effect on nonaccidental mortality [0.55% (0.04, 1.07)], whereas the effect estimates for cardiovascular and respiratory mortality had large confidence intervals. The overall effects in the Latium region were not substantially affected by inclusion or exclusion of Rome.

Table 4 displays PM_{10} effect estimates according to population size. We observed a tendency of higher risks on nonaccidental mortality in smaller spatial units in comparison with larger ones, ranging from 0.76% (−0.35, 1.88) in municipalities with fewer than 5,000 inhabitants to −0.01% (−0.92, 0.90) in cities with more than 40,000 residents (except Rome) (p-value for interaction = 0.103). In contrast, there was a tendency toward stronger PM_{10} effects on respiratory mortality in larger municipalities (p-value for interaction = 0.015).

We also investigated the role of summer and winter temperatures on mortality in the whole Latium region. There was a suggestion of a harmful effect of summer temperature with an IR % (for 1°C increases) equal to 4.74 (−3.85, 14.10), 2.98 (−9.49, 17.17), and 17.2 (−9.53, 51.87) for nonaccidental, cardiovascular, and respiratory mortality, respectively. In contrast, winter temperatures showed no effect on cause-specific mortality.

When we evaluated lagged effects of PM_{10} (lag 0–1) the results were similar in comparison with the main analysis (lag 0) (see Supplemental Material). Finally, modeling the summer and winter temperatures with natural splines with 3 and 2 degrees of freedom, respectively, did not change the effect of PM_{10} on cause-specific mortality (see Supplemental Material).

Table 5 shows the comparison between the main analysis and the sensitivity approach for confounding adjustment (mixed model with random intercepts and slopes, fixed effects model with interaction terms). These models were run for the whole Latium region only. We observed similar estimates for nonaccidental mortality across the three models, ranging from 1.03% (0.49, 1.57) to 0.69% (0.35, 1.04). Cardiovascular mortality displays no longer positive association with PM_{10} in sensitivity analyses, whereas respiratory mortality shows similar effects with the mixed model and higher estimates with the fixed effects model.

Table 2. Annual variation in area-level PM_{10} concentrations and cause-specific mortality rates in Latium region (378 municipalities) and in Rome (155 urban zones): Absolute changes are reported for PM_{10}, percent changes are reported for mortality rates.

			Percentiles			
Latium region	Mean	SD	25th	50th	75th	IQR
Cause-specific mortality						
Nonaccidental	0	48.9	−19.1	−1.1	16.7	35.8
Cardiovascular	0	78.1	−39.7	−3.3	24.4	1.29
Respiratory	0	140	−100	−37.51	41.1	141.1
Particulate matter						
PM_{10} ($\mu g/m^3$)	0.00	2.44	−1.91	−0.39	1.99	3.90
Urban zones of Rome						
Cause-specific mortality						
Nonaccidental	1.33	29.23	−8.11	−0.041	7.80	15.91
Cardiovascular	2.50	37.42	−12.11	−0.17	11.67	23.78
Respiratory	14.23	70.36	−19.96	1.83	29.85	49.81
Particulate matter						
PM_{10} ($\mu g/m^3$)	0	2.96	−2.51	−0.88	2.59	5.1

Discussion

We investigated the relationship between long-term exposure to PM_{10} and cause-specific mortality in the Latium region during the period 2006–2012. Using a variant of difference-in-differences approach, our results provided evidence of a link between PM_{10} and mortality in the study area, with percent increases of mortality rate equal to (for 1-$\mu g/m^3$ increase of PM_{10}) to 0.75%, 0.93%, and 1.42% for nonaccidental, cardiovascular, and respiratory causes, respectively. If we consider the average $PM_{2.5}/PM_{10}$ ratio for the fixed monitors in Rome equal to 0.65, these estimates correspond to a long-term effect of $PM_{2.5}$ equal to 1.16%, 1.43%, and 2.19%, respectively. In addition, we tested alternative approaches for confounding adjustment in sensitivity analyses, obtaining results similar to results using the main approach.

In the last years, many cohort studies investigated the association between long-term exposure to $PM_{2.5}$ and cause-specific mortality worldwide. Hoek et al. performed a systematic review in 2013 (Hoek et al. 2013) in which they meta-analyzed 11 studies reporting the long-term effects of $PM_{2.5}$ on all-cause mortality and 10 studies on cardiovascular mortality. The authors concluded that each increase of $10\,\mu g/m^3$ in $PM_{2.5}$ was associated to an excess risk of 6% (4, 8%), and 11% (5, 16%) for nonaccidental and cardiovascular mortality, respectively. No effect was found for respiratory mortality. In Europe, the ESCAPE Project analyzed the role of long-term exposure to air pollution on several health outcomes in 22 European cohorts. The authors estimated the exposure to several pollutants using land use regression models and examined the association by Cox regression analyses. They estimated hazard ratios (HRs) of 1.04 (1.00–1.09), 1.02 (0.92–1.14), and 0.86 (0.67–1.04) for each $10\,\mu g/m^3$ increase of PM_{10} for nonaccidental, cardiovascular, and nonmalignant respiratory mortality, respectively (Beelen et al. 2014b, 2014a; Dimakopoulou et al. 2014). Their effect estimates were adjusted for environmental and behavioral variables such as smoking status, alcohol consumption, fat intake, and BMI, increasing the importance of the evidence published. Recently, Vodonos et al. (2018) in a systematic review found that for all-cause all-age mortality, a 1-$\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 1.29% increase in all-age all-cause mortality (95% CI: 1.09, 1.50) at a mean exposure of $10\,\mu g/m^3$, which decreased to 1.03% (95% CI: 0.97, 1.11) at a mean exposure of $15.7\,\mu g/m^3$ (the mean level across all studies), and to 0.82% (95% CI: 0.52, 1.12) at $30\,\mu g/m^3$ Vodonos et al. 2018). The estimates for cardiovascular

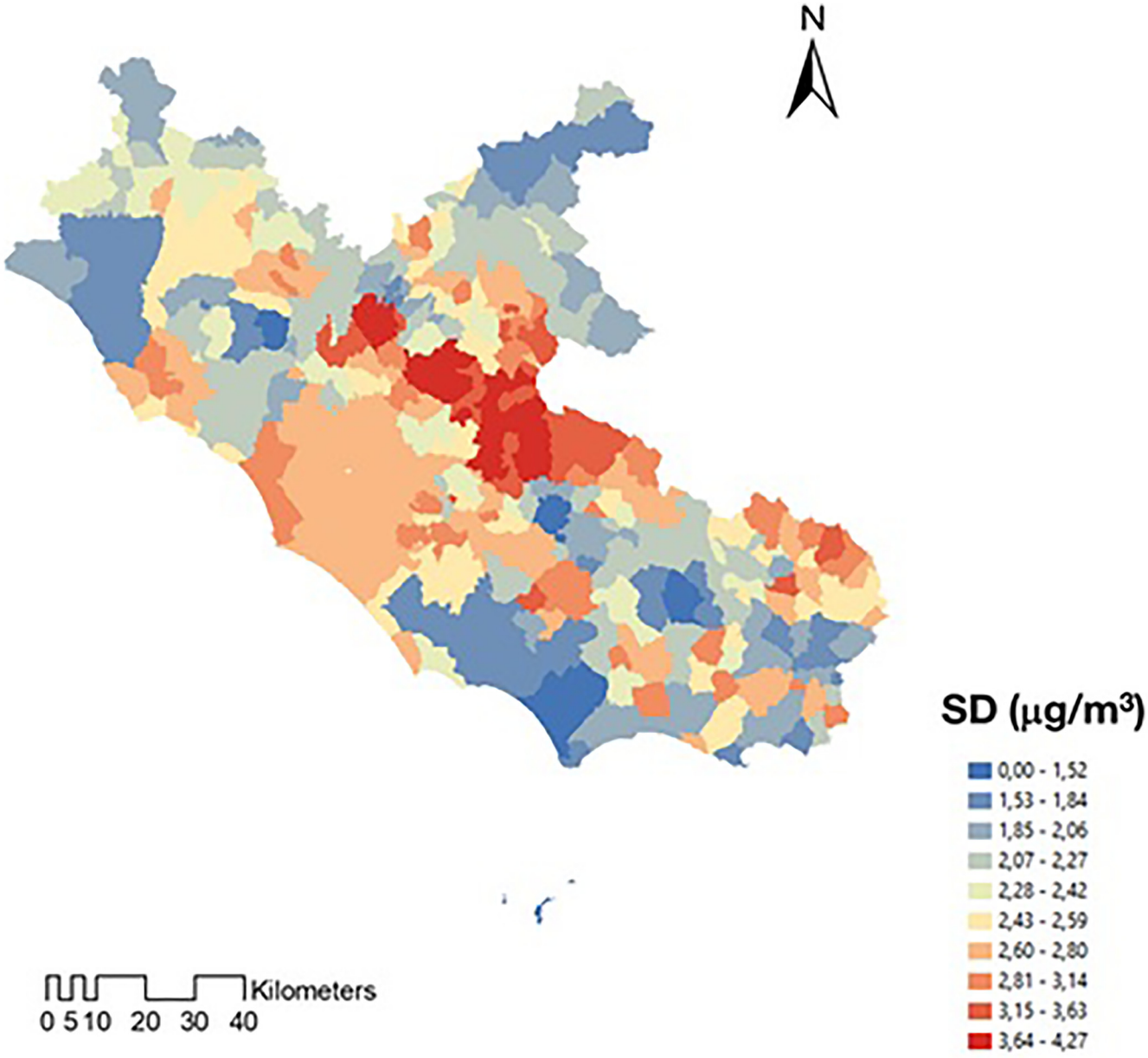

Figure 3. Standard deviation of the annual PM_{10} concentrations for each municipality in the whole region over the period 2006–2012.

and respiratory mortality were 1.46% (95% CI: 1.25, 1.67), and 1.13% (95% CI: 0.85, 1.41), respectively, at a mean exposure of 10 $\mu g/m^3$.

Although quantitative comparisons of the effects of PM_{10} and $PM_{2.5}$ are not entirely appropriate, all the results indicate a clear role of PM exposure on mortality for nonaccidental, cardiovascular, and respiratory causes. We found results very similar to those reported in a recent systematic review conducted by Vodonos et al. It should be noted that there are differences between the methods used in our analysis and those used in previous cohort studies. Most of the available cohort studies used exposure levels at a fixed time and considered only some of the potential individual confounders. In our case, we controlled for each slow-changing variable in time, such smoking status, BMI, socioeconomic status, etc., by design. We also controlled for variables that changed from year to year similarly across the region by design, thus allowing us to control also for unmeasured confounders. Therefore, we have assurance that most unmeasured potential confounders of the previous studies do not confound our results, under the assumption that variations in other variables around location specific means and region specific time trends are not correlated with similar variations in PM_{10}.

Recent studies applied a similar design to investigate the association between particles and mortality. Wang et al. used a variant of difference-in-differences method to assess the effect of long-term exposure to $PM_{2.5}$ on natural causes mortality in New Jersey during the 2004–2009 period (Wang et al. 2016). They assigned an average exposure to $PM_{2.5}$ for each year and for each ZIP code in New Jersey using satellite-based data. They found an IR% of 15.5 (0.8, 32.3) for 10-$\mu g/m^3$ increase of pollutant in the entire population of New Jersey. Although the methods used in that study were similar to our approach, the effect estimates were slightly higher than those that we found. The higher estimates found by Wang et al. could be explained by the pollutant considered. In fact, it is reasonable to expect that PM_{10} would show a lower effect in comparison with that of $PM_{2.5}$ exposure.

Table 3. Associations between long-term exposures to environmental variables and cause-specific mortality. Results are expressed as percent increase of risk and relative 95% confidence intervals (CI) per 1-μg/m^3 increase of PM_{10}.

	Mortality		
Area/cause-specific mortality	IR%	95% CI	
Latium Region			
Nonaccidental	0.75	0.17	1.34
Cardiovascular	0.93	0.03	1.83
Respiratory	1.42	−0.38	3.25
Latium region without Rome			
Nonaccidental	0.57	−0.07	1.22
Cardiovascular	0.59	−0.38	1.57
Respiratory	2.02	0.05	4.04
Rome (155 urbanistic zones)			
Nonaccidental	0.53	−0.05	1.12
Cardiovascular	0.22	−0.64	1.08
Respiratory	0.57	−1.43	2.62

Table 5. Associations between long-term exposures to PM_{10} and mortality for nonaccidental, cardiovascular and respiratory causes over the Latium region by three different modeling approaches: Difference-in-differences (base model), fixed effects model, and mixed model. All results are expressed for 1-μg/m^3 increase in PM_{10}.

Approach	IR%	95%CI	
Difference in differences			
Nonaccidental mortality	0.75	0.17	1.34
Cardiovascular mortality	0.93	0.03	1.83
Respiratory mortality	1.42	−0.38	3.25
Fixed effects model			
Nonaccidental mortality	1.03	0.49	1.57
Cardiovascular mortality	0.59	−0.44	1.63
Respiratory mortality	3.34	0.73	6.02
Mixed model			
Nonaccidental mortality	0.69	0.35	1.04
Cardiovascular mortality	−0.01	−0.45	0.44
Respiratory mortality	0.94	0.83	1.04

However, some other untestable hypotheses could play a role, such as the different study period and study area, different composition of particles, or the economic crisis that struck Europe in 2008 leading to a progressive weakness of the study population (Karanikolos et al. 2013). Kioumourtzoglou et al. used a similar approach to evaluate the effect of long-term exposure to $PM_{2.5}$ in 207 cities in the United States (Kioumourtzoglou et al. 2016). They applied a variant of difference-in-differences method analyzing Medicare enrollees (>65 years old). The authors assigned to each subject of the same city the same annual exposure to $PM_{2.5}$ obtained by city-specific monitors. The results showed a strong positive association with a HR of 1.19 (95% CI: 1.11, 1.28) per 10-μg/m^3 increase of $PM_{2.5}$ related to total mortality. In this case, the comparison with our study is not as immediate because they combined a city-specific exposure with individual information using the counting process extension of the proportional hazards model by Andersen and Gill (Andersen and Gill 1982) instead of the Poisson regressions at city levels applied in our study. However, the two methods are statistically comparable as demonstrated in several frameworks (Peters et al. 2006). In addition, the results displayed similar trends with effect estimates in line with other epidemiological evidence.

Our results suggested that in towns with fewer than 5,000 inhabitants the effect of PM_{10} is higher for nonaccidental mortality and null for respiratory mortality. For bigger cities, we observed an opposite trend with higher effect estimates for respiratory mortality in comparison with other causes. A possible explanation of these results might be chance alone or a different mixture composition of ambient particles in rural areas in comparison with urban areas. Some studies indicate that PM composition in rural areas is characterized mostly by crustal [aluminum (Al), silicon (Si), calcium (Ca), iron (Fe)], and sea-salt components [sodium (Na), chlorine (Cl), magnesium (Mg)], whereas in urban settings, vehicular and industrial constituents represent the greater part of the total composition (Götschi et al. 2005; Viana et al. 2008). Epidemiological evidence showed that some components displayed different effects on cause-specific mortality (Atkinson et al. 2015). For example, Si was associated with nonaccidental mortality in the 15 cohorts of the ESCAPE framework (Beelen et al. 2015). Although these pieces of evidence supported our findings, we were not able to separate the component-specific effects in our study; therefore, this lack of information is an untestable hypothesis in our case.

Our main approach assumed that time trends might be captured adequately by dummies for years, under the assumption that these were uniform in the study region. In the sensitivity analysis, we substantially relaxed such an assumption by adding interactions between linear time trends and municipalities. Results were very similar for natural mortality and comparable for cardiovascular and respiratory outcomes. Although the sensitivity analysis points against substantial residual confounding, we still cannot entirely rule out the existence of omitted covariates, changing differently across years from area to area in a nonlinear way, partially biasing our estimates.

To our knowledge, this investigation is one of the few studies that attempted to estimate the link between long-term exposure to PM_{10} and cause-specific mortality using a difference-in-differences approach. Our approach presents some limitations that we must mention. Apart from temperature, we did not adjust for other spatio-temporal predictors potentially confounding the association under investigation. We could not conceive of any variable displaying different time trends across municipalities and co-varying with annual PM concentrations, other than temperature (which we controlled for) and influenza epidemic. Especially relevant for respiratory mortality, we are not able to control for influenza epidemics, which

Table 4. Associations between long-term exposures to PM_{10} and cause-specific mortality in different type of municipalities (the number of municipalities in each class are reported in brackets) in the Latium region. Results are expressed as percent increase of risk and relative 95% confidence intervals (CIs) per 1-μg/m^3 increase of PM_{10}.

	Mortality											
Effect modifiers	Nonaccidental				Cardiovascular				Respiratory			
Population*	IR%	95% CI		*p*-int	IR%	95% CI		*p*-int	IR%	95% CI		*p*-int
1,000–5,000 (194)	0.76	−0.35	1.88	—	−0.13	−1.63	1.39	—	−0.96	−4.21	2.40	—
5,000–15,000 (123)	0.38	−0.68	1.44	0.286	0.50	−1.03	2.06	0.251	0.99	−2.30	4.40	0.175
15,000–40,000 (108)	0.44	−0.46	1.35	0.302	0.34	−0.95	1.65	0.285	3.57	0.78	6.44	0.007
>40,000 *(31)*	−0.01	−0.92	0.90	0.103	0.50	−0.87	1.89	0.230	2.98	0.24	5.80	0.015

*Size (inhabitants).
Note: *p*-Int, *p*-value for interaction.

might vary both spatially and temporally during the study period and across the study area. In addition, in the extreme case, socioeconomic trends, smoking rates, etc., could be considered under the (unrealistic) hypothesis that they varied differently across the study areas and were also related to annual PM_{10} fluctuations. To critically evaluate our findings, we computed the E-value proposed by VanderWeele and Ding in 2017 (VanderWeele and Ding 2017) to evaluate the effect of potential unmeasured confounding. Briefly, E-value is defined by the authors as "the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the treatment and outcome, conditional on the measured covariates, to fully explain away a specific treatment–outcome association." E-value could be easily calculated by the formula: "$\mathrm{RR} + \mathrm{sqrt}(\mathrm{RR} * (\mathrm{RR} - 1))$." In our case, we estimated an E-value for nonaccidental mortality of 1.39 for the effect estimate and 1.27 for the lower limit of the CI related to the relative risk. This approach means that an unmeasured confounder could explain our estimates away if it were associated with both exposure and outcome with a risk ratio of 1.39. Similarly, it should be associated with both exposure and outcome with a risk ratio of 1.27 to move the CI to include the unity. We consider highly unlikely that such an unmeasured (spatiotemporal) confounder exists. Considering exposure assessment, we know that exposure misclassification can occur from assigning the same yearly averaged PM_{10} in each spatial unit for all residents. In addition, we used average exposure for each city introducing a possible exposure misclassification with relative Berksonian error, which should not bias our estimates but would increase the CIs. Finally, it should be considered that our approach may have a more limited statistical power in comparison with traditional cohort analyses as we have only a limited fluctuation in the PM_{10} concentration around its overall mean over the study period; this lack of power is reflected in the large CIs of the effect estimate for respiratory mortality.

Conclusion

Exposure to ambient air particles represent an important risk factor for human health worldwide; however, the epidemiological findings should be reinforced with alternative study designs and modeling approaches. Our analysis suggests an effect of PM_{10} on cause-specific mortality in the Latium region that is unlikely to be explained by confounding. PM affects mortality not only in urban settings but also in suburban and rural areas. Finally, the difference-in-differences approach might be considered as a good alternative method to estimate an association between long-term exposure to air pollution and mortality.

References

Andersen PK, Gill RD. 1982. Cox's regression model for counting processes: a large sample study. Ann Statist 10(4):1100–1120, https://doi.org/10.1214/aos/1176345976.

Armstrong BG, Gasparrini A, Tobias A. 2014. Conditional Poisson models: a flexible alternative to conditional logistic case cross-over analysis. BMC Med Res Methodol 14:1–6, PMID: 25417555, https://doi.org/10.1186/1471-2288-14-122.

Atkinson RW, Mills IC, Walton HA, Anderson HR. 2015. Fine particle components and health—a systematic review and meta-analysis of epidemiological time series studies of daily mortality and hospital admissions. J Expo Sci Environ Epidemiol 25(2):208–214, PMID: 25227730, https://doi.org/10.1038/jes.2014.63.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48, https://doi.org/10.18637/jss.v067.i01.

Beelen R, Hoek G, Raaschou-Nielsen O, Stafoggia M, Andersen ZJ, Weinmayr G, et al. 2015. Natural-cause mortality and long-term exposure to particle components: an analysis of 19 European cohorts within the Multi-Center ESCAPE Project. Environ Health Perspect 123(6):525–533, PMID: 25712504, https://doi.org/10.1289/ehp.1408095.

Beelen R, Raaschou-Nielsen O, Stafoggia M, Andersen ZJ, Weinmayr G, Hoffmann B, et al. 2014a. Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project. Lancet 383(9919):785–795, PMID: 24332274, https://doi.org/10.1016/S0140-6736(13)62158-3.

Beelen R, Stafoggia M, Raaschou-Nielsen O, Andersen ZJ, Xun WW, Katsouyanni K, et al. 2014b. Long-term Exposure to Air Pollution and Cardiovascular Mortality. Epidemiology 25(3):368–378, https://doi.org/10.1097/EDE.0000000000000076.

Card D, Krueger AB. 1994. Minimum wages and employment: a case study of the fast-food industry in New Jersey and Pennsylvania. Am Econ Rev 84:772–793, https://doi.org/10.2307/2118030.

Cesaroni G, Forastiere F, Stafoggia M, Andersen ZJ, Badaloni C, Beelen R, et al. 2014. Long term exposure to ambient air pollution and incidence of acute coronary events: prospective cohort study and meta-analysis in 11 European cohorts from the ESCAPE Project. BMJ 348:f7412, PMID: 24452269, https://doi.org/10.1136/bmj.f7412.

Chen J, Li C, Ristovski Z, Milic A, Gu Y, Islam MS, et al. 2017. A review of biomass burning: emissions and impacts on air quality, health and climate in China. Sci Total Environ 579:1000–1034, PMID: 27908624, https://doi.org/10.1016/j.scitotenv.2016.11.025.

Cohen AJ, Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, et al. 2017. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet 389(10082):1907–1918, PMID: 28408086, https://doi.org/10.1016/S0140-6736(17)30505-6.

Dee DP, Uppala SM, Simmons AJ, Berrisford P, Poli P, Kobayashi S, et al. 2011. The ERA-Interim reanalysis: configuration and performance of the data assimilation system. QJR Meteorol Soc 137(656):553–597, https://doi.org/10.1002/qj.828.

Di Q, Wang Y, Zanobetti A, Wang Y, Koutrakis P, Choirat C, et al. 2017. Air pollution and mortality in the Medicare population. N Engl J Med 376(26):2513–2522, PMID: 28657878, https://doi.org/10.1056/NEJMoa1702747.

Dimakopoulou K, Samoli E, Beelen R, Stafoggia M, Andersen ZJ, Hoffmann B, et al. 2014. Air pollution and nonmalignant respiratory mortality in 16 cohorts within the ESCAPE project. Am J Respir Crit Care Med 189(6):684–696, PMID: 24521254, https://doi.org/10.1164/rccm.201310-1777OC.

Götschi T, Hazenkamp-Von Arx ME, Heinrich J, Bono R, Burney P, Forsberg B, et al. 2005. Elemental composition and reflectance of ambient fine particles at 21 European locations. Atmos. Environ 39(32):5947–5958, https://doi.org/10.1016/j.atmosenv.2005.06.049.

Hernán MA, Robins JM. 2019. *Causal Inference*. Boca Raton: Chapman & Hall/CRC, forthcoming. https://www.hsph.harvard.edu/miguel-hernan/causal-inference-book/.

Hoek G, Krishnan RM, Beelen R, Peters A, Ostro B, Brunekreef B, et al. 2013. Long-term air pollution exposure and cardio- respiratory mortality: a review. Environ Health 12(1):43, PMID: 23714370, https://doi.org/10.1186/1476-069X-12-43.

Jerrett M, Burnett RT, Beckerman BS, Turner MC, Krewski D, Thurston G, et al. 2013. Spatial analysis of air pollution and mortality in California. Am J Respir Crit Care Med 188(5):593–599, PMID: 23805824, https://doi.org/10.1164/rccm.201303-0609OC.

Karanikolos M, Mladovsky P, Cylus J, Thomson S, Basu S, Stuckler D, et al. 2013. Financial crisis, austerity, and health in Europe. Lancet 381(9874):1323–1331, PMID: 23541059, https://doi.org/10.1016/S0140-6736(13)60102-6.

Kim K-H, Jahan SA, Kabir E, Brown R. 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int 60:71–80, PMID: 24013021, https://doi.org/10.1016/j.envint.2013.07.019.

Kioumourtzoglou M-A, Schwartz JD, James P, Dominici F, Zanobetti A. 2016. PM2.5 and mortality in 207 US cities: modification by temperature and city characteristics. Epidemiology 27(2):221–227, PMID: 26600257, https://doi.org/10.1097/EDE.0000000000000422.

Kloog I, Chudnovsky AA, Just AC, Nordio F, Koutrakis P, Coull BA, et al. 2014. A new hybrid spatio-temporal model for estimating daily multi-year $PM_{2.5}$ concentrations across northeastern USA using high resolution aerosol optical depth data. Atmos Environ (1994) 95:581–590, PMID: 28966552, https://doi.org/10.1016/j.atmosenv.2014.07.014.

Kloog I, Koutrakis P, Coull BA, Lee HJ, Schwartz J. 2011. Assessing temporally and spatially resolved $PM_{2.5}$ exposures for epidemiological studies using satellite aerosol optical depth measurements. Atmos Environ 45(35):6267–6275, https://doi.org/10.1016/j.atmosenv.2011.08.066.

Krewski D, Jerrett M, Burnett RT, Ma R, Hughes E, Shi Y, et al. 2009. Extended follow-up and spatial analysis of the American Cancer Society study linking particulate air pollution and mortality. Respir Rep. Health. Eff. Inst. 5–114; discussion 115–136.

Landrigan PJ, Fuller R, Acosta NJR, Adeyi O, Arnold R, Basu N(N), et al. 2017. The *Lancet* Commission on pollution and health. Lancet 391(10119):462–512, PMID: 29056410, https://doi.org/10.1016/S0140-6736(17)32345-0.

Lepeule J, Laden F, Dockery D, Schwartz J. 2012. Chronic exposure to fine particles and mortality: an extended follow-up of the Harvard six cities study from 1974 to 2009. Environ Health Perspect 120(7):965–970, PMID: 22456598, https://doi.org/10.1289/ehp.1104660.

Makar M, Antonelli J, Di Q, Cutler D, Schwartz J, Dominici F. 2017. Estimating the causal effect of low levels of fine particulate matter on hospitalization. Epidemiology 28(5):627–634, PMID: 28768298, https://doi.org/10.1097/EDE.0000000000000690.

Moolgavkar SH, Chang ET, Watson HN, Lau EC. 2017. An assessment of the Cox proportional hazards regression model for epidemiologic studies. Risk Analysis 38: 777–794, PMID: 28768298, https://doi.org/10.1111/risa.12865.

Peters A, von Klot S, Berglind N, Hörmann A, Löwel H, Nyberg F, et al. 2006. Comparison of different methods in analyzing short-term air pollution effects in a cohort study of susceptible individuals. Epidemiol Perspect Innov 3(1):10, PMID: 16899126, https://doi.org/10.1186/1742-5573-3-10.

Pey J, Querol X, Alastuey A, Forastiere F, Stafoggia M. 2013. African dust outbreaks over the Mediterranean Basin during 2001–2011: PM_{10} concentrations, phenomenology and trends, and its relation with synoptic and mesoscale meteorology. Atmos Chem Phys 13(3):1395–1410, https://doi.org/10.5194/acp-13-1395-2013.

Pope CA, Thun MJ, Namboodiri MM, Dockery DW, Evans JS, Speizer FE, et al. 1995. Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. Am J Respir Crit Care Med 151(3 Pt 1):669–674, PMID: 7881654, https://doi.org/10.1164/ajrccm/151.3_Pt_1.669.

Puett RC, Hart JE, Yanosky JD, Paciorek C, Schwartz J, Suh H, et al. 2009. Chronic fine and coarse particulate exposure, mortality, and coronary heart disease in the Nurses' Health Study. Environ Health Perspect 117(11):1697–1701, PMID: 20049120, https://doi.org/10.1289/ehp.0900572.

Rubin DB. 1991. Practical implications of modes of statistical inference for causal effects and the critical role of the assignment mechanism. Biometrics 47(4):1213–1234, PMID: 1786315, https://doi.org/10.2307/2532381.

Stafoggia M, Schwartz J, Badaloni C, Bellander T, Alessandrini E, Cattani G, et al. 2017. Estimation of daily PM_{10} concentrations in Italy (2006-2012) using finely resolved satellite data, land use variables and meteorology. Environ Int 99:234–244, PMID: 28017360, https://doi.org/10.1016/j.envint.2016.11.024.

Stafoggia M, Zauli-Sajani S, Pey J, Samoli E, Alessandrini E, Basagaña X, et al. 2015. Desert dust outbreaks in Southern Europe: contribution to Daily PM_{10} concentrations and short-term associations with mortality and hospital admissions. Environ Health Perspect 124(4):413–419, PMID: 26219103, https://doi.org/10.1289/ehp.1409164.

Stürmer T, Schneeweiss S, Avorn J, Glynn RJ. 2005. Adjusting effect estimates for unmeasured confounding with validation data using propensity score calibration. Am J Epidemiol 162(3):279–289, PMID: 15987725, https://doi.org/10.1093/aje/kwi192.

Turner H, Firth D. 2015. Generalized nonlinear models in R: An overview of the gnm package. Version 0.9-6. Coventry, UK. https://cran.r-project.org/web/packages/gnm/vignettes/gnmOverview.pdf [accessed 20 June 2018].

VanderWeele TJ, Ding P. 2017. Sensitivity analysis in observational research: introducing the E-value. Ann Intern Med 167(4):268, PMID: 28693043, https://doi.org/10.7326/M16-2607.

Viana M, Kuhlbusch TAJ, Querol X, Alastuey A, Harrison RM, Hopke PK, et al. 2008. Source apportionment of particulate matter in Europe: a review of methods and results. J Aerosol Sci 39(10):827–849, https://doi.org/10.1016/j.jaerosci.2008.05.007.

Vodonos A, Awad YA, Schwartz J. 2018. The concentration-response between long-term $PM_{2.5}$ exposure and mortality; a meta-regression approach. Environ Res 166:677–689, PMID: 30077140, https://doi.org/10.1016/j.envres.2018.06.021.

Wang Y, Kloog I, Coull BA, Kosheleva A, Zanobetti A, Schwartz JD. 2016. Estimating causal effects of long-term PM2.5 exposure on mortality in New Jersey. Environ Health Perspect 124(8):1182–1188, PMID: 27082965, https://doi.org/10.1289/ehp.1409671.

WHO (World Health Organization). International Classification of Diseases, 9th Edition. 1978. http://apps.who.int/iris/handle/10665/39473 [accessed 19 June 2017].

Zanobetti A, Schwartz J. 2009. The effect of fine and coarse particulate air pollution on mortality: a national analysis. Environ Health Perspect 117(6):898–903, PMID: 19590680, https://doi.org/10.1289/ehp.0800108.